Effect of Genetic Selection for Group Productivity and Longevity on Immunological and Hematological Parameters of Chickens

H. W. Cheng,*[,1] S. D. Eicher,* Y. Chen,* P. Singleton,* and W. M. Muir†

*Livestock Behavior Research Unit, USDA-ARS, West Lafayette, Indiana 47907; and †Animal Sciences Department, Purdue University, West Lafayette, Indiana 47907

ABSTRACT A line of White Leghorn chickens was selected for high group productivity and longevity resulting in improved survival and feather score as well as reduced cannibalism and flightiness. Improvements in survival might have also been due to improved immunity. The present study was designed to test the hypothesis that selection for high (HGPS) and low (LGPS) group productivity and survivability also altered immune and hematological parameters. The LGPS line was an intense reverse selected line of the HGPS line at the eighth generation of development. Hens were randomly assigned to individual cages at 17 wk of age. Blood samples were collected from the hens at 21 wk of age. Subsets of T lymphocytes ($CD4^+$, $CD8^+$, and $\gamma\delta$ cells) were measured using flow cytometry. Concentrations of plasma IgG were quantified with western blot analysis and immunoprecipitation assay. Hematological parameters were collected from blood smears.

The HGPS hens had significantly higher percentages of blood lymphocytes and $CD4^+$:$CD8^+$ ratios of circulating T cells ($P < 0.01$) and tended to have more, but not significantly, $\gamma\delta$ T cells ($P = 0.07$) than the LGPS hens. In contrast, the LGPS hens exhibited eosinophilia and heterophilia and greater heterophil:lymphocyte ratios ($P < 0.01$). The concentrations of plasma IgG were also significantly higher in the LGPS hens ($P < 0.01$). These results suggest that genetic selection for group productivity and longevity also alters the immunological and hematological systems of hens. The line difference in regulation of T cells, leukocytes, and production of IgG may suggest that different genes or modes of gene action are involved.

(*Key words*: group selection, well-being, immunology, hematology, chicken)

2001 Poultry Science 80:1079–1086

INTRODUCTION

Cannibalism and aggression are among the major stressors of poultry that cause suffering and death. Current management techniques used to overcome these problems cause the chickens to suffer (beak trimming) or inhibit natural behavior (extremely low light levels); neither management practice is consistent with animal well-being. Selective breeding of chickens for kinder and gentler domestic behavior could provide an alternative approach to address these problems and improve avian well-being.

A selected line of White Leghorn chickens was developed at Purdue University (Muir, 1996; Craig and Muir, 1996; Muir and Craig, 1998) using a genetic selection program termed "group selection@qR that emphasized group productivity and survivability of families housed together in colony cages. Group productivity was based on average rate of lay, and longevity was based on average mortality. Chickens were not beak-trimmed but were kept at 12 hens per cage; lights were at high intensity. The birds genetically selected for high group productivity and survivability (HGPS; previously termed the kinder, gentler bird or KGB) has been shown to improve rate of lay, survival, and feather score as well as reduced cannibalism and flightiness as compared to the unselected control base population from which the line was developed (Craig and Muir, 1996). Compared to control and commercial lines, the HGPS line also had better and faster adaptation to various stressors such as social, handling, cold, and heat stress (Hester et al., 1996b,c). Improvements in survival and greater resistance to stress in the HGPS line might have also been due to improved immunity.

The immune system of chickens, as in other animals, is controlled by genes and genetic-environmental interactions (Gavora, 1993; Siegel, 1995). Several studies have demonstrated that immune response can be directly or indirectly modified by selective breeding, such as antibody

Received for publication August 8, 2000.

Accepted for publication April 9, 2001.

[1]To whom correspondence should be addressed: hwcheng@purdue.edu.

Abbreviation Key: HA = chickens genetically selected for high antibody response to SRBC; H:L = heterophil:lymphocyte; HGPS = chickens genetically selected for high group productivity and survivability; LA = chickens genetically selected for low antibody response to SRBC; LGPS = chickens genetically selected for low group productivity and survivability.

responses to *Escherichia coli* (Yonash et al., 1996; Yunis et al., 2000), *Necrotic enteritis* (Siegel et al., 1993), and SRBC (Siegel and Gross, 1980; Martin et al., 1989), and mitogen-induced T-cell proliferation (Cheng and Lamont, 1988; Kreukniet et al., 1994) as well as phagocyte activities of natural killer cells and macrophages (Cheng and Lamont, 1988; Qureshi and Havenstein, 1994). Differences in immunity may be associated with strain differences in chickens' physical indexes, such as growth rate and egg production, and also affects well-being, such as resistance to infection diseases (Gross and Siegel, 1980; Siegel et al., 1984, 1993; Warner et al., 1987; Bayyari et al., 1997; Nestor et al., 2000). These immune-physical interactions may be affected by prior selection or genotypes involved. For example, divergent selection of chicken lines for high (HA) or low (LA) antibody response to SRBC also divergently affects other physiological characteristics (Siegel and Gross, 1980; Boa-Amponsem et al., 1999; Yang et al., 2000). The HA line chickens has a reduced immunity, higher susceptibility to *E. coli* infection, and lower body weights. Bayyari et al. (1997) also reported that genetic selection for faster growth in turkeys potentially affects immunity. These studies suggest that selective breeding in chickens based on one characteristic, such as IgG or body weight, could affect other genes or systems that associate with physiological characteristics unique to the line in response to stimulation. However, few studies have been designed to investigate the interrelations among genetic factors, domestic behaviors, and immunity in chickens, which is a critical issue for preventing infections and harmful behaviors associated with welfare problems in the poultry industry (Mench 1992; Craig and Swanson, 1994; Dietert et al., 1994; Siegel, 1995; Bumstead, 1998).

Changes in chicken behaviors and performance, resulting from selective breeding, are reflected in changes in their physiological homeostasis, including immune system (Sandi et al., 1992). However, except for the study by Hester et al. (1996a) comparing humoral immune response to SBRC challenge in the HGPS line and control lines, genetically induced changes in immunity of the HGPS line and chickens selected for low group productivity and survivability (LGPS) have not been well documented. The objectives of the present study were to physiologically characterize HGPS and LGPS lines and to determine the effect of genetic selection on the immunological and hematological parameters.

MATERIALS AND METHODS

Genetic Lines

The ninth generation of the HGPS and LGPS lines were used as the genetic material for this research. The HGPS line was produced from hens of 12 multiple-hen cages with the highest group productivity (number of eggs) and lowest mortality at 72 wk of age (after 52 wk of production), along with their full- and half-sib brothers. The LGPS line was developed from hens of 12 cages with the lowest group productivity and highest mortality, along with their full- and half-sib brothers. The line differences in productivity and survivability (Cheng et al., 2001), and the detail of the selection technology and rearing program, has been previously reported (Muir, 1996).

Pullets of each genetic line were reared under the same conditions, i.e., hatched, vaccinated for Marek's and Newcastle diseases, and maintained using standard management practices in raised wire cages up to 17 wk of age. At 17 wk of age, hens from each genetic line were randomly assigned to individual cages, each providing 1,085 cm^2 per hen. The hens of both lines were not beak-trimmed and were kept in multiple-hen cages in the same room during the entire experimental period. Feed and water were provided ad libitum. Overhead lights were on daily from 0700 until 1900 h initially and were increased by 15 min/wk. The photoperiod was 13 h daily when the study was performed.

Chicken care guidelines were in strict accordance with the rules and regulations set by Federation of Animal Science Societies (Craig et al., 1999). Experimental protocols were approved by the institutional Animal Care and Use Committee at Purdue University. Efforts were made to minimize animal suffering and the number of animals used.

Blood Sampling

Based on the previous investigations (Craig and Muir, 1996) the major categories of behavioral adaptations were stable after 3 wk of housing. At 21 wk of age, or after 4 wk of housing, 24 hens each from the HGPS and LGPS lines were sampled. A 5-mL blood sample was collected from the brachial vein within 2 min of removing the hen from its cage. The heparinized blood was stored on ice. Blood samples were centrifuged at $700 \times g$ for 15 min at 20 C. Plasma was used for analysis of IgG, and the white cells were used for analysis of subsets of T lymphocytes.

Quantitative Analysis of Blood Parameters

Duplicate blood smears were prepared from unheparinized blood samples by using a cover glass technique and were stained within 1 h of preparation with Wright's stain (Campbell, 1988). A double-blind design was used for the cell counts. Two hundred leukocytes on each duplicate slide were examined at 2,000× magnification. Heterophils, lymphocytes, monocytes, basophils, and eosinophils were identified based on their characteristics described by Campbell (1988), from which the heterophil to lymphocyte (H:L) ratio was calculated (Gross and Siegel, 1983).

Flow Cytometry Analysis for Subsets of T Cells

Flow cytometry analyses of T lymphocytes, including $CD4^+$, $CD8^+$, and $\gamma\delta$ cells, were performed with slight modification as previously described (Eicher-Pruiett et al., 1992; Boeker et al., 1999). Peripheral blood lymphocytes were isolated from the buffy coat layer of the centrifuged blood

sample. The cell number was counted using a Coulter Z1 cell counter,[2] and then cells were resuspended in Rose Park Memorial Institute medium 1640[3] at 1×10^6 cells/mL. A 200-μL aliquot of the cell suspension of each sample was added to separate tubes for the T cell determination by using directly conjugated fluorescein isothiocyanate and phycoerythrin antibodies.[4,5] Labeled cells were measured using a Coulter XL MCL flow cytometer[2] with a 488 nm air-cooled argon laser for excitation, a 525 band pass for fluorescein isothiocyanate labels, and a 575 nm band pass for phycoerythrin detection.

Immunoprecipitation Assay for Concentrations of Plasma IgG

Concentrations of blood IgG were measured in triplicate using a chicken IgG immuno-diffusion assay kit[6] by following the protocol supplied by the company. Five microliters of 10× diluted sample plasma and two known standard Solutions A and B were separately added to wells of IgG gel plates containing specific antiserum to chicken IgG. Two wells containing no test serum were included on each plate as negative controls. After incubation at 37 C for 48 h, the precipitation ring was measured, and concentrations were determined as compared to the standards.

Western Blot Analysis for IgG

Western blots were analyzed in duplicate with slight modification of the protocol as described previously (Chamanza et al., 1999; McNeill et al., 1999). Ten microliters of a 10× diluted sample plasma, commercially purified chickens IgG,[7] and molecular weight marker were denatured at 96 C for 3 min and electrophoresed constantly in a 10% SDS-polyacrylamide gel. Separated proteins on the gels were examined after Coomassie blue staining. For immunoblotting, the separated proteins were transferred to nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline and then incubated in primary antisera (rabbit anti-chicken IgG[7]), followed by horseradish peroxidase conjugated secondary antibody (Goat anti-rabbit IgG[7]) in Tris-buffered saline containing 0.2% Tween-20.

Visualization of the site of antigen-antibody complex was carried out with enhanced chemiluminescence solution and hyperfilm[8] according to the procedure supplied with the kit. Blots were quantified by computer-assisted video densitometry[9] as described previously (McNeill et al., 1999). The results were expressed as a concentration index that was calculated by dividing the optical density of samples by the optical density of commercially purified chicken IgG.

Statistical Analysis

The experimental design was completely randomized with genetic lines as the main effect. Results were assessed statistically using one-way ANOVA with the cages as the experimental unit. Percentage data for the leukocyte counts was transformed to arc sine (Steel and Torrie, 1980). Actual and transformed values for physiological variables were assessed using ANOVA. Because statistical trends were similar for the transformed and untransformed data, the untransformed results are presented.

RESULTS

Genetic Selection-Induced Alterations in Hematological Parameters

The HGPS hens, as compared to the LGPS hens, had a significantly higher percentage of blood lymphocytes (Table 1; $P < 0.01$) but a significantly lower percentage of heterophils ($P < 0.01$). As a result of the shift in the differential counts, the H:L ratio was significantly lower in the HGPS line than in the LGPS line. The LGPS hens also exhibited a greatly higher percentage of eosinophils ($P < 0.01$) than the HGPS hens. There were no significant differences between the lines in the percentages of monocytes and basophils.

Genetic Selection-Induced Alterations in Subsets of T Cells

The phenotypes of T lymphocytes were measured using flow cytometry (Figure 1). There were no significant differences of the percentage of $CD4^+$ T cells between the lines (Table 2; $P = 0.1$), but the percentages of $CD8^+$ T cells were significantly lower in the HGPS hens compared to the LGPS hens ($P < 0.01$). As a result of the shift in the differential subsets of T cells, the ratio of $CD4^+$ to $CD8^+$ T cells was significantly higher in the HGPS line than in the LGPS line ($P < 0.01$). Compared to the LGPS hens, the hens of the HGPS line tended to have a higher percentage of $\gamma\delta$-positive T cells (16.1 vs. 12.2; $P = 0.07$).

Genetic Selection-Induced Alterations in Plasma IgG Concentrations

A band (~67 kDa) was present in the SDS-PAGE of all plasma samples examined (Figure 2a). The band was later identified as IgG, which matched the band prepared from commercially purified chicken IgG (Figure 2). The optical density for each IgG band on the immunoblots was measured. Results showed that LGPS hens had significantly higher concentrations of plasma IgG than the HGPS hens, which was confirmed by immunoprecipitation assay (Table 3; $P < 0.01$).

[2]Coulter Inc., Kennesaw, GA 30144.
[3]Life Technology, Inc., Frederick, MD 21704.
[4]Southern Biotechnology, Inc., Birmingham, AL 35226.
[5]B&D PharMingen, San Diego, CA 92121.
[6]Cardlotech Services, Inc., Louisville, KY 40205.
[7]Jackson Immunoresearch Laboratories, West Grove, PA 19390.
[8]Amersham Pharmacia Biotech., Piscataway, NJ 08855.
[9]Kodak digital science 1D image analyzer software, Eastman Kodak Company, Rochester, NY 14650.

TABLE 1. Genetic selection-induced alterations in the differential leukocyte counts in hens

Line[1]	Heterophils[2] (H)	Lymphocytes (L)	H:L ratio (× 100)	Monocytes	Eosinophiles	Basophils
HGPS	10.7[b] ± 1.1[3]	83.4[a] ± 1.3	13.0[b]	2.6 ± 0.4	1.7[b] ± 0.2	1.6 ± 1.1
LGPS	20.4[a] ± 1.8	72.3[b] ± 1.8	29.4[a]	2.1 ± 0.4	3.8[a] ± 0.4	1.4 ± 0.2
HGPS:LGPS	53%	115%	44%	124%	45%	114%

[a,b]Means within a column with no common superscript differ significantly ($P < 0.01$).

[1]The HGPS and LGPS lines were selected from the control line based on high and low productivity and survivability resulting from cannibalism and flightiness, respectively.

[2]Four hundred leukocytes were counted from duplicate blood smears for each hen.

DISCUSSION

The present study demonstrated that there are line differences in the populations of T cells and leukocytes as well as in the production of IgG in the HGPS and LGPS chickens. Those immunological and hematological systems are related to unique coping ability of the line to novel environments and resistance to stressors (Craig and Muir, 1996; Hester et al., 1996a,b,c). The present results are consistent with previous studies that have demonstrated that genetic differences in productivity and social behaviors are associated with individual difference in immunity (Petitto et al., 1994; Hessing et al., 1995; Maes, 1995; Perna et al., 1997; Hawken et al., 1998).

The ratio of $CD4^+$ to $CD8^+$ T cells may be used as an indictor of cell-mediated immune response. Previous studies have reported that the normal ratio of $CD4^+$ to $CD8^+$ T cells should be greater than 1.5; otherwise, cellular immune mechanisms are greatly impaired (Levinson and Jawetz, 1996) and survivability is damaged (Reid and Tervit, 1995). Compared to the LGPS hens, the HGPS hens may have a more efficient cell-mediated immunity, because the $CD4^+$ to $CD8^+$ ratio of circulating T cells was much higher in the HGPS hens than that in the LGPS hens. In addition, the percentage of several cell types involved in the cell-mediated immunity tended to be greater in the hens of the HGPS line than in the LGPS line, including monocytes (124%) and $\gamma\delta$ T cells (131%). Similar to the present data, Bayyari et al. (1997) reported that in turkeys, genetic selection for faster growth affects cell-mediated immunity. These results support the hypothesis that selective breeding in chickens based on one indicator could affect other physiological characteristics by involving different genes or modes of gene actions.

Heterophilia and the H:L ratio have been used as physiological indicators of stress in evaluation of chicken respon-

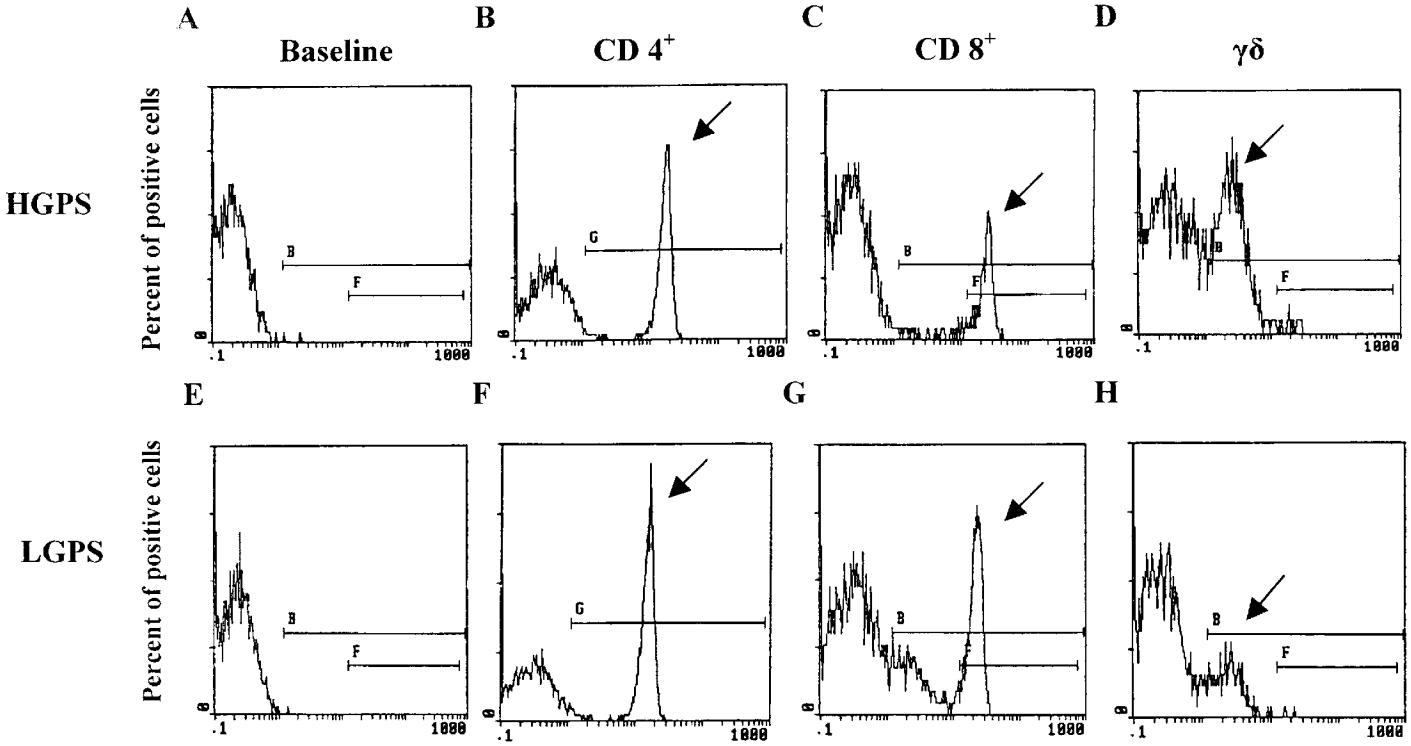

FIGURE 1. Examples of the surface expression of the $CD4^+$, $CD8^+$, and $\gamma\delta$ molecule on T cells from the HGPS (A, B, C and D) and LGPS (E, F, G and H) hens. A, E) background cells only; B, F) $CD4^+$ T cells; C, G) $CD8^+$ T cells; D, H) $\gamma\delta$ T cells. The peak (arrow) in each graph refers to percentage of positive cells. HGPS and LGPS = hens genetically selected for high and low group productivity and survivability resulting from reduced cannibalism and flightiness, respectively.

TABLE 2. Genetic selection-induced alterations in the subpopulations of T cells in hens

Group[1]	$CD4^+$ cells (% positive)	$CD8^+$ cells (% positive)	$CD4^+$:$CD8^+$	$\gamma\delta$ cells (% positive)
HGPS	33.0 ± 2.1[2]	18.2[b] ± 1.8	1.9[a]	16.1 ± 2.1
LGPS	29.3 ± 3.3	25.8[a] ± 1.5	1.1[b]	12.2 ± 2.8
HGPS:LGPS	113%	71%	173%	131%

[a,b]Means within a column with no common superscript differ significantly ($P < 0.01$).

[1]The HGPS and LGPS lines were selected for high and low productivity and survivability resulting from cannibalism and flightiness, respectively.

siveness to novel environments (Gross and Siegel, 1983; Beuving et al., 1989; Maxwell, 1993, Hester et al., 1996c). The HGPS hens had less heterophils and a lower H:L ratio, which suggests that they could have a greater adaptive capability to stress than the hens of the LGPS line. This result is consistent with previous studies that have documented that HGPS hens exhibited better feathering score, lower mortality, and higher reproduction in a socially crowded environment (Craig and Muir, 1996). In addition, the HGPS hens showed improved adaptation to various stressors such as social, handling, cold, and heat stress when compared to the control birds (Hester et al., 1996a,c).

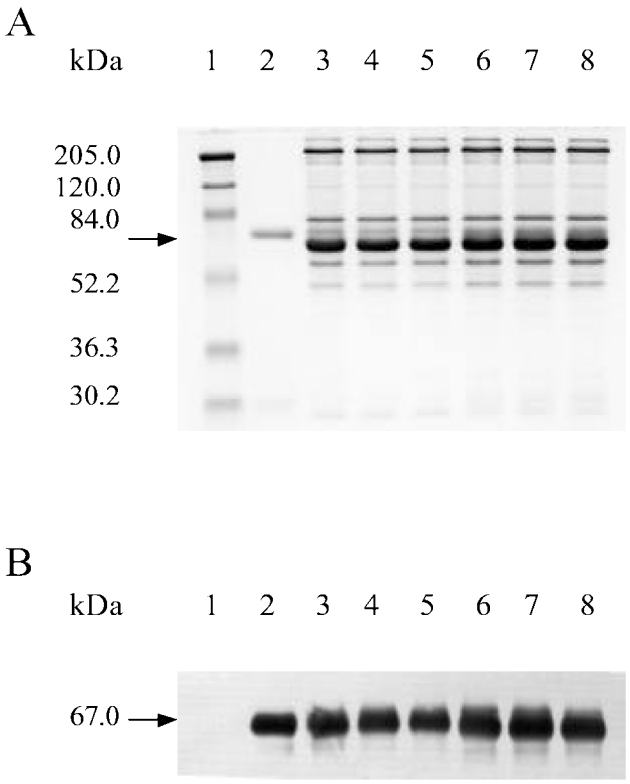

FIGURE 2. Quantity analysis of chicken IgG on SDS-PAGE. A) separated proteins on the gels were stained with Coomassie blue; B) separated protein on the gels were detected by the Western blot analysis plus enhanced chemiluminescence. Lane 1, prestained broad-range molecular weight marker; Lane 2, commercial chicken IgG; Lanes 3 to 5, samples from HGPS hens; Lanes 6 to 8, samples from LGPS hens. Note the band from samples was identical with the band prepared with commercial chicken IgG, and size of IgG was identified approximately at 67 kDa (arrow). HGPS and LGPS = hens genetically selected for high and low group productivity and survivability resulting from reduced cannibalism and flightiness, respectively.

The present findings suggest that the H:L ratio and heterophilia could be used as indicators for genetic selection of chicken strains with higher resistance to stress. However, caution is advised, although selecting for high group productivity resulted in altered H:L ratio, the reverse may not be true, i.e., selection for altered H:L ratio may not change group productivity in the desired direction.

Retention of normal levels of circulating eosinophils is associated with resistance to stress (Woolaston et al., 1996; Hohenhaus et al., 1998), and changes in blood eosinophils appear as a genotypic or phenotypic hallmark of physiological and psychological stress reactions (Malyshev et al., 1993; Hohenhaus et al., 1998). Their hypothesis is consistent with the present findings that eosinophilia was exhibited in the LGPS hens but not in the HGPS hens. These results also suggest that number of eosinophils could be used as an indicator of animal well-being.

Circulating eosinophils has been used as a genetic selection criterion for resistance of sheep to trichostrongyle parasites (Woolaston et al., 1996) and used as a phenotypic marker of resistance to nematode parasites (Hohenhaus et al., 1998). Selection-induced different regulation of eosinophils in the present chicken lines could be associated with the selection-related alterations of the neuroendocrine immune system. A correlation has been established between alterations in blood eosinophils, corticosterone levels, and catecholamine metabolism (Malyshev et al., 1985). As such, injection of adrenocorticotropin hormone induces eosinophilia in black steers (Ishizaki and Kariya, 1999) and hens (Gray et al., 1989). Eosinopenia induced by handling and sampling can be blocked by adrenalectomy and hypophysectomy in rats (Treloar, 1977).

Plasma IgG was upregulated in the LGPS hens but not in the HGPS hens. This result is consistent with the belief that alterations in IgG synthesis are a common response

TABLE 3. Genetic selection-induced alteration in blood concentration of IgG

Group[1]	Western blot analysis (concentration index)	Immunoprecipitation assay (mg/mL)
HGPS	1.2[b] ± 0.07[2]	7.5[b] ± 0.07
LGPS	1.4[a] ± 0.06	10.8[a] ± 0.7
HGPS/LGPS	86%	69%

[a,b]Means within a column with no common superscript differ significantly ($P < 0.01$).

[1]The HGPS and LGPS line were selected for high and low productivity and survivability resulting from cannibalism and flightiness, respectively.

of B cells to genetic selection in birds and mammals (Hammerling et al., 1973; Siegel and Gross, 1980; Siegel et al., 1982; Van der Zijpp et al., 1983; Clark et al., 1996). The mechanisms underlying inherent higher IgG concentrations in the LGPS hens could be related to the lower ratio of $CD4^+$ to $CD8^+$ T cells. Although $CD4^+$ T cells induce B cell production of antibodies (T cell-dependent response), antibody production can be suppressed by inhibitory lymphokines released by $CD4^+$ T cells (Levinson and Jawetz, 1996). In addition, the higher levels of IgG can occur without involvement of T cells (T cell-independent response); memory B cells are not required (Levinson and Jawetz, 1996). Without an immune challenge, it cannot be assumed that the LGPS hens have fewer memory B cells, but Boa-Amposem et al. (1999) have shown that in response to booster inoculation with SBRC, the chickens of their HA line exhibited significantly lower immunological memory than chickens of the LA line.

The present study showed there were a negative correlation between total IgG concentrations and productivity and survivability in the present strains. Higher concentrations of IgG were associated with lower productivity and survivability in the LGPS hens. Similarly, several negative relationships have been found between genetic lines from different species, including body weight, feeding efficiency, and egg production in chickens (Siegel and Gross, 1980; Siegel et al., 1982; Van der Zijpp et al., 1983; Gross and Siegel, 1988), milk production in dairy cattle (Detilleux et al., 1991; Weigel et al., 1992), and levels of emotional stress in mice (Ozherelkov et al., 1985). In chickens selected for high and low antibody response to SRBC following immune challenge with Marek's disease virus, mortality was higher in the HA line than the LA line (Tamaki 1981; Okada and Yamamoto, 1987; Martin et al., 1989), and the HA line was more sensitive to *E. coli*, *Pasteurella multocida*, or both than the LA line (Gross et al., 1980; Dunnington et al., 1991). The reason for the differing regulation of IgG in our selected lines remains unclear, but it could be the same cellular mechanisms as those reported for the HA and LA lines.

The results indicate that selection for total antibody production in the HA and LA lines altered the number of antibody-producing cells or different subset cells involved (Martin et al., 1989; Sarker et al., 1999). For example, The HA line, compared to the LA line, had a reduced effectiveness of macrophages (Gross and Siegel, 1988), lower proliferative T cells (Shanks et al., 2000), and lesser immunological memory (Boa-Amponsem et al., 1999). The changes in T-cell subsets in LGPS hens, compared to the HGPS hens, such as a lower $CD4^+$ to $CD8^+$ ratio of T cells, may produce suitable conditions for the production of low affinity and self-reactive antibodies (Doria et al., 1997).

It remains unclear whether the different immunological and hematological characteristics indicate that cell-mediated immunity and IgG production are controlled by independent genes in the present lines, but previous studies have indicated that cell- and humoral-mediated immunity are regulated differently among different chicken genotypes (Gehad et al., 1999). Kreukniet et al. (1994) also found that concanavalin A-induced T-cell proliferation was higher in the low line than in the high line, the lines being bi-directionally selected for low or high antibody produced against SRBC. Moreover, in their low line, they found that the high T-cell activity was related to a genetically induced severe decline in antibody responses to several T-cell-dependent antigens (Kreukniet et al., 1992).

Similar genetic differences regulating chicken cell- and humoral-mediated immunities were also identified by Van der Zijpp et al. (1983) and Lamont and Smyth (1984a,b). Although identification of genetic factors that differentiate the dynamic regulation of the hens' immunity in the present selected lines is unclear, previous studies have shown that several cellular mechanisms could be involved in the regulation. It could be related to selection-induced changes in physiological functions of the neuroendocrine system, including hypothalamus-pituitary-adrenal and sympathetic-medullary-adrenal axes. These changes subsequently influence the animal's immungenetic expression (Dohms and Metz, 1991).

Data from a parallel study had shown that dopamine and catecholamines were regulated differently in the HGPS and LGPS hens (Cheng et al., 2001). Differing regulation of these monoamines have been related to stress-induced alterations in behavioral adaptation and productive performance (Summers et al., 1997). Through binding to their receptors within immune organs and cells (Plaut, 1987), enhanced blood dopamine and catecholamines in the LGPS line may have a suppressive effect on immunity. Previous studies have shown dopamine and catecholamines as well as corticosterone suppress proliferation of T and B cells and natural killer cell responsiveness to antigens and inhibit cytokine production (Brown-Borg et al., 1993; Hessing et al, 1995; Dunn, 1996; Wilkie and Mallard, 1999). In addition, differential regulation of immunity could be related to selection-induced morphofunctional changes of lymph organs and immunological cells that have been identified in previously selected chickens. For example, the LA chickens have larger thymuses and smaller spleens and bursas of Fabricius than the HA chickens (Ubosi et al., 1985).

In conclusion, the present investigation demonstrates that there are line differences in the regulation of subsets of T cells and leukocytes as well as production of IgG. These results suggest that selection for group productivity and longevity has also altered the hens' immunological and hematological systems. These differences may also be involved in controlling productivity and longevity and in regulating adaptability to novel environments and resistance to stressors. The HGPS and LGPS lines could used as models to investigate the molecular and cellular mechanisms underlying effects of genetic factors on physiological functions of neuroimmunoendocrine communication in controlling productivity and longevity as related to domestic behaviors.

ACKNOWLEDGMENTS

We thank J. Johnson for technical assistance in the flow cytometry assay, G. Dillworth in blood smear preparation

and slide reading, and J. Neilson of the Animal Care and Use Committee at Purdue University for guiding in the care and use of birds in the study.

REFERENCES

Bayyari, G. R., W. E. Huff, N. C. Rath, J. M. Balog, L. A. Newberry, J. D. Villines, J. K. Skeeles, N. B. Anthony, and K. E. Nestor, 1997. Effect of the genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. Poultry Sci. 76:289–296.

Beuving, G., R. B. Jones, and H. J. Blokhuis, 1989. Adrenocortical and heterophil/lymphocyte responses to challenge in hens showing short or long tonic immobility reactions. Br. Poult. Sci. 30:175–184.

Boa-Amponsem, K., E. A. Dunnington, K. S. Baker, and P. B. Siegel, 1999. Diet and immunological memory of lines of White Leghorn chickens divergently selected for antibody response to sheep red blood cells. Poultry Sci. 78:165–170.

Boeker, M., R. Pabst, and H. J. Rothkotter, 1999. Quantification of B, T and null lymphocyte subpopulations in the blood and lymphoid organs of the pig. Immunobiology 201:74–87.

Brown-Borg, H. M., H. G. Klemcke, and F. Blecha, 1993. Lymphocyte proliferative responses in neonatal pigs with high or low plasma cortisol concentration after stress induced by restraint. Am. J. Vet. Res. 54:2015–2020.

Bumstead, N., 1998. Genetic resistance to avian viruses. Rev. Sci. Technol. 17:249–255.

Campbell, T. W., 1988. Avian Hematology and Cytology. Iowa State University Press Ames, IA.

Chamanza, R., M. J. Toussaint, A. M. van Ederen, L. van Veen, C. Hulskamp-Koch, and T. H. Fabri, 1999. Serum amyloid A and transferrin in chicken. A preliminary investigation of using acute-phase variables to assess diseases in chickens. Vet. Q. 21:158–162.

Cheng, H. W., G. Dillworth, and W. M. Muir, 2001. Effect of group selection for productivity and longevity on blood concentrations of serotonin, catecholamine, and corticosterone of chickens. Poultry Sci. 80:(in press).

Cheng, S., and S. J. Lamont, 1988. Genetic analysis of immunocompetence measures in a White Leghorn chicken line. Poultry Sci. 67:989–995.

Clark, J., T. Bourne, M. P. Salaman, M. H. Seifert, and D. A. Isenberg, 1996. B lymphocyte hyperactivity in families of patients with systemic lupus erythematosus. J. Autoimmun. 9:59–65.

Craig, J. V., W. F. Dean, G. B. Havenstein, K. K. Kruger, K. E. Nestor, G. H. Purchase, P. B. Siegel, and G. L. van Wicklen, 1999. Guidelines for poultry husbandry. Pages 55–66. *in*: Guide for the care and use of agricultural animals in agricultural research and teaching. Federation of Animal Science Societies. Savoy, IL.

Craig, J. V., and W. M. Muir, 1996. Group selection for adaptation to multiple-hen cages: beak-related mortality, feathering, and body weight responses. Poultry Sci. 75:294–302.

Craig, J. V., and J. C. Swanson, 1994. Review: welfare perspectives on hens kept for egg production. Poultry Sci. 73:921–938.

Detilleux, J. C., A. E. Freeman, and L. D. Miller, 1991. Comparison of natural transmission of bovine leukemia virus in Holstein cows of two genetic lines selected for high and average milk production. Am. J. Vet. Res. 52:1551–1555.

Dietert, R. R., K. A. Golemboski, and R. E. Austic, 1994. Environment-immune interactions. Poultry Sci. 73:1062–1076.

Dohms, J. E., and A. Metz, 1991. Stress—mechanisms of immunosuppression. Vet. Immunol. Immunopathol. 30:89–109.

Doria, G., G. Biozzi, D. Mouton, and V. Covelli, 1997. Genetic control of immune responsiveness, aging and tumor incidence. Mech. Ageing Dev. 96:1–13.

Dunn, A. J., 1996. Psychoneuroimmunology, stress and infection. Pages 25–46 *in*: Psychoneuroimmunology, Stress, and Infection. H. Friedman, T. W. Klein and A. L. Friedman, Eds. CRC Press, Boca Raton, FL.

Dunnington, E. A., P. B. Siegel, and W. B. Gross, 1991. Escherichia coli challenge in chickens selected for high or low antibody response and differing in haplotypes at the major histocompatibility complex. Avian Dis. 35:937–940.

Eicher-Pruiett, S. D., J. L. Morrill, F. Blecha, J. J. Higgins, N. V. Anderson, and P. G. Reddy. 1992. Neutrophil and lymphocyte response to supplementation with vitamins C and E in young calves. J. Dairy Sci. 75:1635–1642.

Gavora, J. S., 1993. Genetic control of disease and disease resistance in poultry. Pages 231–241 *in*: Manipulation of Avian Genome. R. J. Etches and A. M. Verrinder-Gibbins, ed. CRC Press, Boca Raton, FL.

Gehad, A. E., M. M. Mashaly, H. S. Siegel, E. A. Dunnington, and P. B. Siegel, 1999. Effect of genetic selection and MHC haplotypes on lymphocyte proliferation and interleukin-2 like activity in chicken lines selected for high and low antibody production against sheep red blood cells. Vet. Immunol. Immunopathol. 68:13–24.

Gray, H. G., T. J. Paradis, and P. W. Chang, 1989. Physiological effects of adrenocorticotropic hormone and hydrocortisone in laying hens. Poultry Sci. 68:1710–1713.

Gross, W. B., and P. B. Siegel, 1980. Effects of early environmental stresses on chicken body weight, antibody response to RBC antigens, feed efficiency, and response to fasting. Avian Dis. 24:569–579.

Gross, W. B., and H. S. Siegel, 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27:972–979.

Gross, W. B., and P. B. Siegel, 1988. Environment-genetic influences on immunocompetence. J. Anim. Sci. 66:2091–2094.

Gross, W. B., P. B. Siegel, R. W. Hall, C. H. Donnermuth, and R. T. DuBoise, 1980. Production and persistence of antibodies in chickens to sheep erythrocytes. 2. Resistance to infectious diseases. Poultry Sci. 59:205–210.

Hammerling, G. J., T. Masuda, and H. O. McDevitt, 1973. Genetic control of the immune response. Frequency and characteristics of antigen-binding cells in high and low responder mice. J. Exp. Med. 137:1180–1200.

Hawken, R. J., C. W. Beattie, and L. B. Schook, 1998. Resolving the genetics of resistance to infectious diseases. Rev. Sci. Tech. 17:17–25.

Hessing, M. J., G. J. Coenen, M. Vaiman, and C. Renard, 1995. Individual differences in cell-mediated and humoral immunity in pigs. Vet. Immunol. Immunopathol. 45:97–113.

Hester, P. Y., W. M. Muir, and J. V. Craig, 1996a. Group selection for adaptation to multiple-hen cages: humoral immune response. Poultry Sci. 75:1315–1320.

Hester, P. Y., W. M. Muir, J. V. Craig, and J. L. Albright, 1996b. Group selection for adaptation to multiple-hen cages: hematology and adrenal function. Poultry Sci. 75:1295–1307.

Hester, P. Y., W. M. Muir, J. V. Craig, and J. L. Albright, 1996c. Group selection for adaptation to multiple-hen cages: production traits during heat and cold exposures. Poultry Sci. 75:1308–1314.

Hohenhaus, M. A., M. J. Josey, C. Dobson, and P. M. Outteridge, 1998. The eosinophil leucocyte, a phenotypic marker of resistance to nematode parasites, is associated with calm behaviour in sheep. Immunol. Cell Biol. 76:153–158.

Ishizaki, H., and Y. Kariya, 1999. Effects of peripheral blood polymorphonuclear leukocyte function and blood components in Japanese black steers administered ACTH in a cold environment. J. Vet. Med. Sci. 61:487–492.

Kreukniet, M. B., N. Gianotten, M. B. Nieuwland, and H. K. Parmentier, 1994. In vitro T cell activity in two chicken lines divergently selected for antibody response to sheep erythrocytes. Poultry Sci. 73:336–340.

Kreukniet, M. B., A. J. van der Zijpp, and M. G. Nieuwland, 1992. Effects of route of immunization, adjuvant and unrelated antigens on the humoral immune response in lines of chickens

selected for antibody production against sheep erythrocytes. Vet. Immunol. Immunopathol. 33:115–127.
Lamont, S. J., and J. R. Smyth, Jr., 1984a. Effect of selection for delayed amelanosis on immune response in chickens. 1. Antibody production. Poultry Sci. 63:436–439.
Lamont, S. J. and J. R. Smyth, Jr., 1984b. Effect of selection for delayed amelanosis on immune response in chickens. 2. Cell-mediated immunity. Poultry Sci. 63:440–442.
Levinson, W., and E. Jawetz, 1996. Medical Microbiology and Immunology. Appleton and Lance, Stamford, CT.
Maes, M., 1995. Evidence for an immune response in major depression: a review and hypothesis. Prog. Neuropsychopharmacol. Biol. Psychiatry 19:11–38.
Malyshev, V. V., V. A. Petrova, and B. N. Manukhin, 1985. Changes in the level of eosinophils, corticosterone and catecholamine metabolism in the dynamics of emotional-painful stress. Bull. Exp. Biol. Med. 99:267–269.
Malyshev, V. V., L. S. Vasil'eva, and V. V. Kuz'menko, 1993. The interrelationship between inflammation and the stress reaction. Bull. Exp. Biol. Med. 116:348–349.
Martin, A., W. B. Gross, and P. B. Siegel, 1989. IgG and IgM responses in high and low antibody-selected lines of chickens. J. Hered. 80:249–252.
Maxwell, M. H., 1993. Avian blood leucocyte responses to stress. World's Poult. Sci. J. 49:34–43.
McNeill, T. H., N. Mori, and H. W. Cheng, 1999. Differential regulation of the growth-associated proteins, GAP-43 and SCG-10, in response to unilateral cortical ablation in adult rats. Neuroscience 90:1349–1360.
Mench, J. A., 1992. Introduction: applied ethology and poultry science. Poultry Sci. 71:631–633.
Muir, W. M., 1996. Group selection for adaptation to multiple-hen cages: Selection program and direct responses. Poultry Sci. 75:447–458.
Muir, W. M., and J. V. Craig, 1998. Improving animal well-being through genetic selection. Poultry Sci. 77:1781–1788.
Nestor, K. E., J. W. Anderson, and R. A. Patterson, 2000. Genetics of growth and reproduction in the turkey. 14. Changes in genetic parameters over thirty generations of selection for increased body weight. Poultry Sci. 79:445–452.
Okada, I., and Y. Yamamoto, 1987. Immunocompetences and Marek's disease resistance in three pairs of chicken lines selected for different immunological characters. Poultry Sci. 66:769–773.
Ozherelkov, S. V., V. V. Vargin, and B. F. Semenov, 1985. Effect of experimental information and emotional stress on the primary immune response of different strains of mice. Zh. Mikrobiol. Epidemiol. Immunobiol. 8:43–46.
Perna, F. M., N. Schneiderman, and A. LaPerriere, 1997. Psychological stress, exercise and immunity. Int. J. Sports Med. 18 (Suppl 1):S78–83.
Petitto, J. M., D. T. Lysle, J. L. Gariepy, and M. H. Lewis, 1994. Association of genetic differences in social behavior and cellular immune responsiveness: effects of social experience. Brain Behav. Immun. 8:111–122.
Plaut, M., 1987. Lymphocyte hormone receptors. Annu. Rev. Immunol. 5:621–669.
Qureshi, M. A., and G. B. Havenstein, 1994. A comparison of the immune performance of a 1991 commercial broiler with a 1957 random bred strain when fed "typical" 1957 and 1991 broiler diets. Poultry Sci. 73:1805–1812.
Reid, G. M., and H. Tervit, 1995. Sudden infant death syndrome (SIDS): immunoglobulins and hypoxia. Med. Hypotheses 44:202–206.
Sandi, C., J. Borrell, and C. Guaza, 1992. Behavioral factors in stress-induced Immunomodulation. Behav. Brain Res. 48:95–98.
Sarker, N., M. Tsudzuki, M. Nishibori, and Y. Yamamoto, 1999. Direct and correlated response to divergent selection for serum immunoglobulin M and G levels in chickens. Poultry Sci. 78:1–7.
Shanks, N., R. J. Windle, P. A. Perks, M. S. Harbuz, D. S. Jessop, C. D. Ingram, and S. L. Lightman. 2000. Early-life exposure to endotoxin alters hypothalamic-pituitary-adrenal function and predisposition to inflammation. Proc. Natl. Acad. Sci. U S A. 97:5645–5650.
Siegel, H. S., 1995. Gordon Memorial Lecture. Stress, strains and resistance. Br. Poult. Sci. 36:3–22.
Siegel, H. S., H. L. Marks, J. W. Latimer, and R. L. Wilson, 1984. Plasma constituents and body weights of Japanese quail (Coturnix coturnix japonica) selected for twelve generations for plasma cholesterol responses to adrenocorticotropin. Poultry Sci. 63:222–233.
Siegel, P. B., and W. B. Gross, 1980. Production and persistence of antibodies in chickens to sheep erythrocytes. 1. Directional selection. Poultry Sci. 59:1–5.
Siegel, P. B., W. B. Gross, and J. A. Cherry, 1982. Correlated responses of chickens to selection for production of antibodies to sheep erythrocytes. Anim. Blood Groups Biochem. Genet. 13:291–297.
Siegel, P. B., A. S. Larsen, C. T. Larsen, and E. A. Dunnington, 1993. Research note: resistance of chickens to an outbreak of necrotic enteritis as influenced by major histocompatibility genotype and background genome. Poultry Sci. 72:1189–1191.
Steel, R. G. D., and J. H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., Inc., New York, NY.
Summers, T. R., A. L. Hunter, and C. H. Summers, 1997. Female social reproductive roles affect central monoamines. Brain Res. 767:272–278.
Tamaki Y., 1981. Selection for chicken serum IgG levels and disease resistance (in Japanese). Annu. Rep. Nat. Inst. Anim. Ind. (JPN) 20:97–104.
Treloar, O. L., 1977. Hormonal regulation of rapid eosinopenia in the rat. Lab. Anim. Sci. 27:635–640.
Ubosi, C. O., W. B. Gross, and P. B. Siegel, 1985. Divergent selection of chickens for antibody production to sheep erythrocytes: age effect in parental lines and their crosses. Avian Dis. 29:150–158.
Van der Zijpp, A. J., K. Frankena, J. Boneschanscher, and M. G. Nieuwland, 1983. Genetic analysis of primary and secondary immune responses in the chicken. Poultry Sci. 62:565–572.
Warner C. M., D. L. Meeker, and M. F. Rothschild, 1987. Genetic control of immune responsiveness: a review of its use as a tool for selection for disease resistance. J. Anim. Sci. 64:394–406.
Weigel, K. A., A. E. Freeman, M. E. Kehrli, Jr., J. R. Thurston, and D. H. Kelley, 1992. Relationship of in vitro immune function with health and production in Holstein cattle. J. Dairy Sci. 75:1672–1679.
Wilkie, B., and B. Mallard, 1999. Selection for high immune response: an alternative approach to animal health maintenance? Vet. Immunol. Immunopathol. 72:231–235.
Woolaston, R. R., P. Manueli, S. J. Eady, I. A. Barger, L. F. Le Jambre, D. J. Banks, and R. G. Windon, 1996. The value of circulating eosinophil count as a selection criteria for resistance of sheep to trichostrongyle parasites. Int. J. Parasitol. 26:123–126.
Yang, N., C. T. Larsen, T. E. A. Dunnington, P. A. Geraert, P. M. Picard, and P. B. Siegel, 2000. Immune competence of chicks from two lines divergently selected for antibody response to sheep red blood cells as affected by supplemental vitamin E. Poultry Sci. 79:799–803.
Yonash, N., G. Leitner, R. Waiman, E. D. Heller, and A. Cahaner, 1996. Genetic differences and heritability of antibody response to Escherichia coli vaccination in young broiler chicks. Poultry Sci. 75:683–690.
Yunis, R., A. Ben-David, E. D. Heller, and A. Cahaner, 2000. Immunocompetence and viability under commercial conditions of broiler groups differing in growth rate and in antibody response to *Escherichia coli* vaccine. Poultry Sci. 79:810–816.